



Microbial evaluation of market milk and milk-products of Mizoram, India with special reference to *Staphylococcus aureus*

JULLY G TIWARI¹, SANDEEP P CHAUDHARY², HARISH KUMAR TIWARI³, TAPAN KUMAR DUTTA⁴, PRASANTA SAIKIA⁵ and PRAGATI HAZARIKA⁶

Central Agricultural University, Selesih, Aizawl, Mizoram 796 014 India

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Millions of people throughout the world become victims of staphylococcal food poisoning, one of the major milk-borne diseases, every year when enterotoxin produced by this organism is ingested with food (Minor and Marth 1972). Their growing resistance to the commonly used antimicrobial agents has been a serious problem for both physicians and veterinarians (Tiwari and Tiwari 2007). India has 31% vegetarians in its populace and they depend heavily on milk and milk-products for wholesome nutrition (Yadav and Kumar 2006). Practically vegetarianism is synonymous with lacto-vegetarianism in India. India is the largest producer of milk in the world and its tropical climate provides conducive environment for outbreaks of gastrointestinal infections. Hence, a project of one year duration was undertaken to study the microbial quality of milk and milk-products and also the incidence and antimicrobial susceptibility pattern of *Staphylococcus aureus* isolated from milk and milk-products of Mizoram.

This study was conducted during Aug 2008–09 in Mizoram. Market milk samples (105) were collected in sterile screw-capped plastic tubes in approximately 10 ml quantity aseptically. Likewise, 100 milk-products, viz. *burfi*, *paneer*, ice-cream and *kulfi* were collected (25 each) in approximately 50 g quantity in sterilized containers from various shops of Mizoram. The milk samples collected under aseptic conditions were thoroughly mixed and subjected to serial

tenfold dilution. The milk-product samples were processed within 2 to 3 h of collection. From each sample 10 g was weighed aseptically and transferred into 90 ml of sterile 0.1% peptone water (diluent), so as to form 1 in 10 (10^{-1}) dilution and homogenized at 8000 rpm for 2–3 min to extricate the bacteria from the sample into the diluent. From 10^{-1} dilution serial decimal dilution were prepared up to 10^{-8} (10^{-8}) by transferring 1 ml volumes into 9 ml of diluent. The selected dilutions were further processed for estimation of bacterial load as per APHA (1976). The counts per gram of sample were estimated by multiplying the mean colony forming unit (CFU) count of the sample in duplicate plate with the dilution factor. The total viable counts (TVC) in CFU/g were transformed to logarithms ($\log_{10}$ counts) for further interpretations. The total viable counts (TVC) were subjected to standard statistical analysis to calculate mean $\pm$ SE.

The milk and milk-product samples were thoroughly mixed and streaked onto Baird-Parker agar plates. These plates were then incubated at 37°C for 36 h. After incubation black colonies surrounded by a whitish precipitate zone appeared on the Baird-Parker agar plates which were typical of *Staphylococcus aureus*. These colonies were sub-cultured on nutrient agar plates for purification. The organisms were identified on the basis of morphological, cultural and biochemical characteristics (Buchanan and Gibbons 1974). Antimicrobial susceptibility test was carried out by agar disc diffusion method (Matsen and Barry 1974) using 12 commercially available antimicrobial agents.

The study on milk and milk-products of Mizoram revealed that more than 50% of the samples were unacceptable in respect of total viable count (TVC). The average count of 6.87 (Table 1) from milk is in agreement with findings of Donnelly *et al.* (1968) and Karimuribo *et al.* (2005), who have reported total count of 3×10^6 and 8.9×10^5 to 1.0×10^7 , respectively from milk samples. The average count of 7.29 to 7.79 in milk-products can be compared with the counts

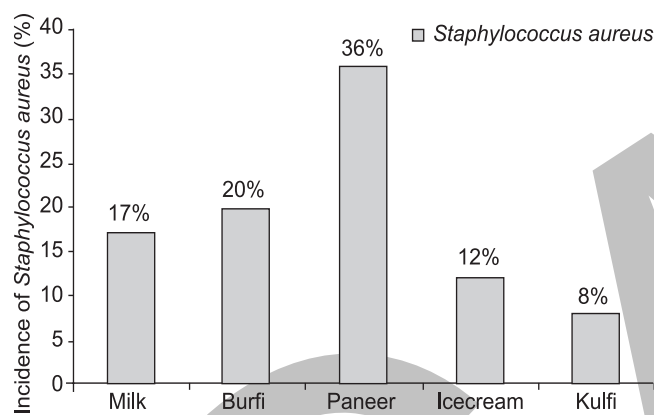
Present address: ¹Assistant Professor (e mail: vetjgt@gmail.com), Department of Veterinary Public Health and Epidemiology; ⁴Associate Professor, Department of Veterinary Microbiology; ⁵Assistant Professor, Department of Livestock Products Management; ⁶ Assistant Professor, Department of Livestock Products Technology.

²Associate Professor, Department of Veterinary Public Health, Nagpur Veterinary College, Seminary Hills, Nagpur, Maharashtra 440 006 India.

³Veterinary Officer, Garhwal Scouts, Joshimath, C/O 56 APO, India.

Table 1. Total viable count of milk and milk-product samples of Mizoram, India

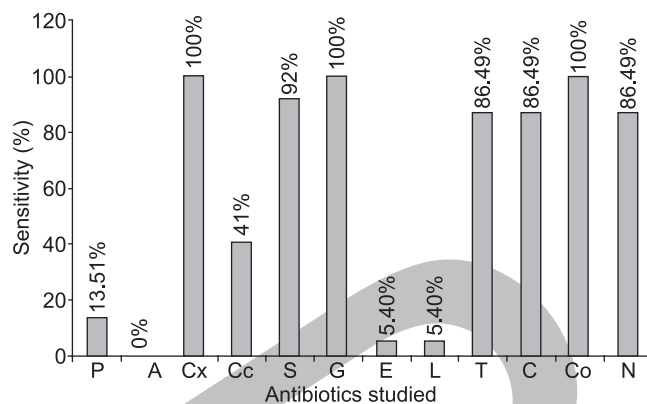
Sample	Total no. of sample analysed	No. of samples in acceptable range	Total plate count bacteria/ml/g		Plate count (Log ₁₀) mean±SE
			Min. Log ₁₀	Max. Log ₁₀	
Milk	105	44 (41.90%)	4.52	9.44	6.87±0.14
Burfi	25	9 (36%)	6.62	9.30	7.78±0.23
Paneer	25	3 (12%)	6.43	8.48	7.79±0.16
Ice cream	25	2 (8%)	5.49	8.31	7.29±0.13
Kulfi	25	12 (48%)	6.87	8.25	7.56±0.16

Fig 1. Incidence of *Staphylococcus aureus* in milk and milk-products of Mizoram, India.

of 3×10^2 to 9.7×10^{10} CFU/ml as reported by Vaishnavi *et al.* (2001). The higher microbial load of the samples can be attributed to unhygienic production of milk by the small-scale dairy farmers and inappropriate storage temperature..

The overall isolation of *Staphylococcus aureus* (Fig.1) from 205 milk and milk-product samples were 37 (18.09%). Similar findings were reported by Gupta (1973,1976), Jayappa *et al.* (1977), Yadav *et al.* (1985), Koehler (1984) and Dedykhina and Lyakhova (1988). The presence of *Staphylococcus aureus* in significant numbers in milk (17.14%) act as potential health hazard to the consumers. As *Staphylococcus aureus* is the principal organism causing mastitis in animals. So, the udder of cows suffering from mastitis can be major reservoir for *Staphylococci*. The wide variation of *Staphylococcus aureus* in milk-product samples (Fig.1) of Mizoram might be due to preparation, storage and transport. The high level of contamination of *burfi* and *paneer* with *Staphylococcus aureus* found in the present study might be due to products being handled frequently and stored at temperature suitable for the growth of this organism.

The antimicrobial study of the strains of *Staphylococcus aureus* (Fig.2) showed complete resistance (100%) against ampicillin. This finding is similar with Buragohain (1988). Ampicillin is one of the very frequently used antibiotics in dairy animals and human. Therefore, the finding of complete

Fig 2. Sensitivity pattern of *Staphylococcus aureus* against 12 commercially used antibiotics.

resistance against ampicillin in the present study was not surprising. The strains showed identical sensitivity against erythromycin and lincomycin of 5.40%. Erythromycin is a frequently used antibiotic in human medicine. Although, lincomycin is not commonly used in human medicine, Clindamycin, a very closely related antibiotic to lincomycin, is often used to treat anaerobic infections in humans. Development of resistance by any bacteria to clindamycin automatically develops resistance to lincomycin too (Dutta and Devriese 1981).

Thus it can be concluded that milk and milk-product samples sold in the various markets of Mizoram, India are of poor microbial quality in respect of total viable count. The heavy *Staphylococcus aureus* contamination in milk and milk-product samples can be attributed to poor hygienic conditions, fault in manufacturing, handling and inappropriate storage temperature. This organism can be a very good source of food poisoning cases. As the occurrence of milk and milk-product associated food poisoning are very common among the children and elderly people of Mizoram, improvement in dairy products preparation and storage has been recommended.

SUMMARY

Samples of milk (105) and 100 samples of milk-products, viz. *burfi*, *paneer*, ice cream and *kulfi* comprising 25 each were collected from various shops of Mizoram, India. The incidence of pathogenic *Staphylococcus aureus* were recorded as 17, 20, 36, 12, and 8, respectively for milk, *burfi*, *paneer*, ice-cream and *kulfi*. The overall incidence of pathogenic *Staphylococcus aureus* in milk and milk-products was estimated as 18.09%. The antimicrobial sensitivity test revealed that all the strains of *Staphylococcus aureus* were sensitive (100%) against cloxacillin, cotrimoxazole and gentamicin. The strains showed complete resistance (100%) against ampicillin. The strains showed identical sensitivity against erythromycin and lincomycin of 5.40%. The sensitivity of *Staphylococcus aureus* to other chemotherapeutic agents in decreasing order was

streptomycin (91.89), chloramphenicol (86.49%), nitrofurantoin (86.49%), tetracycline (86.49%), cephotaxime (40.54%) and penicillin (13.51%).

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